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DNA topology and transcription

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Chromatin is a complex assembly that compacts DNA inside the nucleus while providing the necessary level of accessibility to regulatory factors conscripted by cellular signaling systems. In this superstructure, DNA is the subject of mechanical forces applied by variety of molecular motors. Rather than being a rigid stick, DNA possesses dynamic structural variability that could be harnessed during critical steps of genome functioning. The strong relationship between DNA structure and key genomic processes necessitates the study of physical constrains acting on the double helix. Here we provide insight into the source, dynamics, and biology of DNA topological domains in the eukaryotic cells and summarize their possible involvement in gene transcription. We emphasize recent studies that might inspire and impact future experiments on the involvement of DNA topology in cellular functions.

Keywords: DNA topoisomerases, DNA topological domain, DNA topology, torsional stress, transcription

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Introduction

By definition, the formation of a topological domain requires topologically constrained DNA. The constraint might result from a pair of physical clamps attaching the DNA at specialized sites or from restrained rotation of one strand of the double helix around the other in the viscous cellular environment.[1] Consequently, DNA within a topological domain can be subjected to torsional tension or supercoiling (**Fig. 1A**, right). Topological constraints in chromatin DNA could arise at many hierarchical levels of genome organization.

Many cellular processes including transcription require the cumulative action of multiple protein-DNA interactions. Once a protein binds to DNA, it can serve as a foundation for further protein-DNA complexes setting up a composite net of topologically constrained DNA, but unfortunately we know very little about this phenomenon.[2] At the basic level of chromatin organization the pattern of DNA topology seems simpler. In the cell nucleus, strong and repetitive interactions between DNA and histones form nucleosomes. The nucleosome is the fundamental unit of chromatin in which 147 DNA base pairs are wrapped around a core of histone proteins.[3] Short stretches of naked DNA—linker regions—connect adjacent nucleosomes. Linker DNA ranges between 20 and 90 bp in length and varies between different species and even within a single cellular genome.[4,5] Because the exit and entry sites of a linker region are fixed by the embracing nucleosomes, the linker DNA could be viewed as topological domain of the same length (**Fig. 1B**). Torsional tension of DNA between adjacent nucleosomes, together with linker length, defines the spatial orientation, boundaries, and interactions between nucleosomes and may even orchestrate their structural rearrangements.[6]

Domain boundaries can also result from the tracking of molecular machinery (i.e., transcription complexes) along the DNA double helix (**Fig. 1C**). As originally conceived in the twin-domain-model, during transcriptional elongation, DNA rotation is prevented by frictional force or anchoring of the polymerase complex to a nuclear structure.[7]

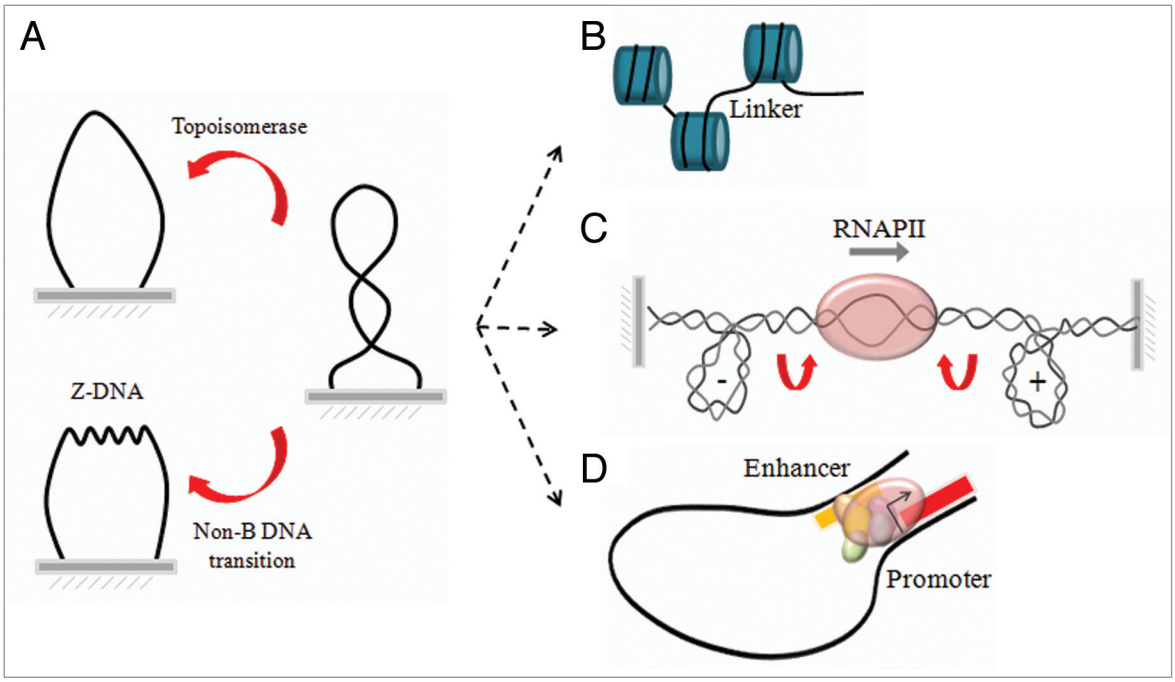

Figure 1. Topological domains of DNA during cellular processes. (**A**) By definition a topological domain requires topologically constrained DNA ends. Within a domain, genetic transactions can distort the structure of the double helix and generate supercoilings, eventually removed through the relaxation activity of DNA topoisomerases or the transitions of regular B-DNA into non-B DNA conformations (Z-DNA is shown as example). Topological domains can be identified at many hierarchical levels: (**B**) in the linker region between two adjacent nucleosome; (**C**) during transcriptional elongation where the RNA polymerase constitutes a moving node between fixed ends; and (**D**) during enhancer-promoter interaction.

Un-twisting or over-twisting of DNA corkscrewing through the transcribing RNA polymerase results in the wave of dynamic supercoiling which might be exploited to participate in the regulation of a variety of DNA transactions.[8]

In eukaryotic genomes, large number of elements dispersed in linear genome has an impact on transcriptional control. The current belief is that the main mechanism by which these regulatory elements communicate with their target genes is through chromatin looping.[9,10] Using approaches based mainly on the chromosome conformation capture technique (3C),[11] looping constraints are inferred from interaction frequencies between a point of interest and distal loci of the genome (**Fig. 1D**). Looping brings distal elements into close spatial proximity to each other and generates the so-called "topological domain".[12] However, this term may often be misleading: the loop-organization of genomes does not necessary means that DNA of any given "topological domain" is indeed topologically isolated from their neighborhood. Co-localization can be the result of specific constraints between two loci mediated by protein complexes, or it can be a nonspecific result of chromatin fiber packing in the crowded nucleus.

The proteins that bind and manipulate the DNA of a true topological domain necessarily impart torsional stress which redistributes over constrained DNA regions and has a significant influence on global conformation of double helix. DNA topoisomerases—the enzymes which modulate the topological state of genome—are vital and common to all cellular organisms.[13,14] Topoisomerases introduce a transient break in the DNA backbone and allow the release of mechanical stress from the double helix (**Fig. 1A**, left). In addition, when the stress is high enough, it can be released as strain in a variety of DNA structural transition: from B-DNA to Z-DNAs, cruciforms, quadruplexes, etc.[15-18]

Proper gene expression occurs largely through the regulation of RNA polymerase transcription which arises at multiple stages: nucleosome remodeling and promoter selection, early transcription from the melting of DNA by the transcriptional machinery through the release of RNA polymerase from promoter-proximal pause sites, transcript elongation, promoter-enhancer interaction, 3-D genomic architecture, and antisense activity.[19-25] Although there are no steps purely managed by DNA topology, a growing body of evidence indicates that DNA topology is an important player in the biochemical team. In this review our goal is to dissect the key regulatory steps of transcription, highlighting the importance and the consequences of topological changes within different types of genomic domains.

DNA Topology and Initiation of Transcription

Cells use transcription factors to regulate transcription initiation by RNA polymerase II, but transcription requires disruption of a repressive chromatin context at promoters. Torsional tension of DNA could have profound effect on the local operation of DNA binding proteins, including nucleosomes,

components of general transcriptional machinery, and a variety of chromatin organizers. Nucleosomes are dynamic structures that must be modified to allow transcription. It has been shown that chromatin remodeling factors facilitate these conformational dynamics.[21,26] Both in vitro and in vivo studies have revealed that as pre-requisite for gene activation all Snf2p-related nucleosome remodelers generate torsional tension in the DNA.[27] This DNA topology-based mechanism provides a powerful way to disrupt repressive chromatin structures at the promoters. ATPase generated torque is even capable of inducing transitions of regular B-DNA into non-B DNA conformations.[28] Transcription from the promoters that form Z-DNA may require the establishment of a boundary between a nucleosome and Z-DNA,[29] as was shown for the promoter of CSF1 gene that adopts a Z-DNA conformation when transcription is activated by BRG1.[30]

Core histone rearrangement and/or acetylation by factors such as p300/CBP release some of the negative supercoils previously restrained by the nucleosomes.[31] Consequently, the changes induced in the chromatin structure result in the topological stress of linker DNA that may create a friendlier neighborhood for general transcription factors and RNA polymerase. Topological coupling between chromatin remodeling and transcription factors binding was inferred from the observation that interaction of the TATA-box Binding Protein is enhanced for supercoiled DNA.[32] Accordingly, pre-initiation complex formation and transcription initiation are assisted by torsional stress in vitro.[33] This view is also supported by studies that suggested the importance of histone acetylation for transcriptional initiation, but not for elongation, and showed that this modification is often observed on the flanking regions of genes.[34,35] In addition, topoisomerase activity is directly required for efficient disassembly of nucleosomes at active promoters.[36]

The next step in transcription initiation is the transition of the closed complex into an open complex, with local melting of the promoter DNA. This transition depends on the recruitment of TFIIH which contains the ATPase activity required for promoter opening and transition to the open complex.[37] To investigate how RNA polymerase responds to DNA supercoiling, a single-molecule approach monitored polymerase-dependent DNA unwinding in torsionally stressed DNA.[38] It was demonstrated that the DNA topology influences the rate of formation and stability of the open complex. Negative supercoiling weakens base stacking interactions, thereby promoting the formation of the transcription bubble. Thus the open complex formation is controlled at least in part by DNA topology. Accordingly, in vitro transcription is more efficient and does not require TFIIH if the promoter resides in a negatively supercoiled plasmid.[33] Therefore, optimal transcription necessitates a delicate balance between topology of the promoters and transcriptional output. Indeed, recent genome-wide experiments suggest that cells have elaborate mechanisms to coordinate the rates of transcription and the DNA relaxing activity of topoisomerases to adjust supercoiling in the promoter regions of differentially expressed genes.[39,40]

The mechanics of promoter melting by TFIIH subunits has been a long standing question. Because the ATPase has helicase activity in vitro, it was thought that TFIIH directly separates the two strands of the DNA double helix to form the transcriptional bubble.[41] However, recent studies have shown that TFIIH translocates along the double helix and rotates DNA inside of the topologically closed domain established by TFIIH-TFIIE interaction.[42] This rotation results in DNA torsional stress, which is relieved by promoter melting.[37,43] Thus, the key step in the formation of an open complex is based on DNA topology properties which could help to explain TFIIH-related diseases.[44]

After promoter melting, RNA polymerase initiates RNA synthesis at the transcription start site. Increasing evidence show that RNA polymerase II often halts just after promoter clearance, typically ~20–60 nucleotides downstream.[45,46] In fact, recent genome-wide studies suggest that paused RNA polymerase II is a common feature of gene regulation, especially in development.[47] Release of the paused polymerase is emerging as one of the major mechanisms of gene control.[48] Promoter escape depends on the phosphorylation of the large subunit of RNA polymerase II by TFIIH and other general transcription factors such as the Mediator complex and the positive transcription elongation factor b (P-TEFb).[23] However the full mechanism(s) of polymerase pausing and release is still pending. In the recent high resolution mapping of DNA supercoiling near promoters of a human cell line it was shown that paused genes have higher level of torsional tension localized near their transcription start sites compared with elongating genes.[39] Moreover, experiments with topoisomerase inhibitors imply that the level of this tension is topoisomerase I dependent. These observations are in good accordance with the finding that treatment of cells with topoisomerase I inhibitor elicits a redistribution of RNA polymerase along transcribed genes and enhances the escape from pausing sites.[40,49,50] This suggests that the pause-release of the transcriptional machinery may be influenced by the specific DNA supercoiling balance at promoters. The precise mechanism still remains to be established, however one might hypothesize that the activity of pause-regulated factors and/or the processivity of transcriptional machinery are coupled to local DNA topology around the pause site. Indeed, in a refined set of experiments monitoring RNA polymerase translocating in real-time along supercoiled DNA, it was shown that the arrest of polymerase imposed by accumulating supercoils was relieved upon release of the opposing torque.[51]

DNA Topology and Transcription Elongation

Transcription elongation results in severe topological perturbation of DNA.[52] During the movement of the transcriptional machinery along chromatin template, nucleosomes should be redistributed, thus releasing or rearranging the negative supercoils they constrain. Additionally, screwing

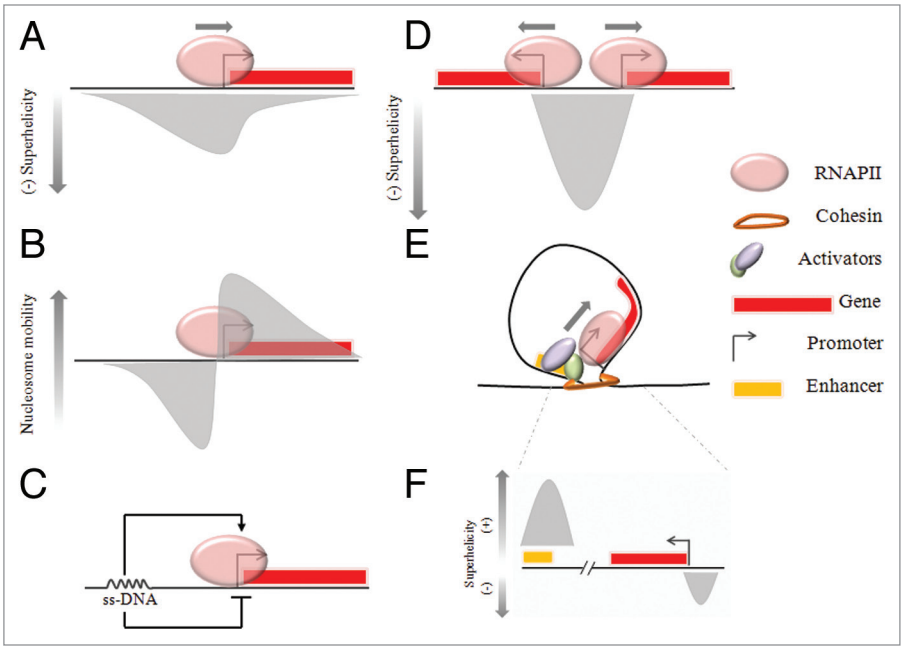

Figure 2. Transcription is associated with dynamic perturbation of DNA. (**A**) Negative DNA supercoiling occurs at upstream promoter regions of every transcribed gene. (**B**) Nucleosome mobilization potential is differentially affected upstream and downstream of transcribing RNA polymerase. (**C**) Non-B DNA formed as result of ongoing transcription has the capacity to regulate the promoter output in real-time. (**D**) The activity of divergent closely juxtaposed promoters may be mechanically coupled through dynamic supercoiling. (**E**) Enhancer transcription could be required to generate torsional stress which results in reorganization of local chromatin structure and favors enhancer-promoter communication.

DNA through the RNA polymerase imposes dynamic torsional tension on the template (**Fig. 2A**).[51,53] In vitro, even a single nucleosome strongly inhibits elongation of transcription, but evidently this is not a problem in vivo.[54] It is crucial to understand how RNA polymerase traverses through nucleosome arrays to account for the efficiency of transcription in vivo.

Nucleosome mobilization in front of polymerase is a possible mechanism to achieve high elongation efficiency. A surprising observation is that gene activation decreases nucleosome occupancy along the full body of the gene well before the first polymerase even approaches the end of the gene.[55] Furthermore, in vitro negative supercoiling promotes nucleosome assembly, whereas positive supercoiling inhibits it.[56] The reorganization of a multiprotein complex such as a chromatin fiber by the diffusion of the chromatin remodelers should be a rather slow process. Torsional tension in DNA can propagate in a much faster fashion suggesting that positive supercoiling generated in front of elongating RNA polymerase could help to de-condense chromatin for more efficient transcription.[57] Though poly-ADP ribose polymerase (PARP) was shown to mediate this process because it can associate with topoisomerases, involvement of DNA topology remains a viable mechanistic consideration for gene body opening.[58,59] To investigate how nucleosomes respond to different topological environments, nucleosomes on DNA under tension and torque were studied using an elegant single-molecule approach. Dramatic loss of H2A/H2B dimers was observed at the physiologically relevant level of positive torsional tension suggesting that DNA topology can be a potent regulator of nucleosomes mobilization during transcription.[60] A recent in vivo study also supports this idea (**Fig. 2B**, right). Using a sequencing-based assay to determine DNA torsional states with high resolution, it was shown that accumulation of positive supercoiling results in increased nucleosome turnover within gene bodies.[40]

RNA polymerase is an highly processive enzyme with velocity in vivo up to 70 bases per second.[61] If elongation proceeds without rotation of the transcriptional apparatus, 1 negative supercoil should be generated for each turn of double helix and almost 1500 supercoils per averaged 15 kb human gene length.[62,63] Therefore it is reasonable to wonder how transcription might exploit this enormous level of supercoiling or whether this is just a by-product, eventually cleaned-up by topoisomerases. However, topoisomerases do not instantaneously relax these supercoils and high torsional stress could drive transition of B-DNA into non-B conformation at the susceptible sequences.[39,53] In fact, identification of non-B DNA structures upstream of the active promoters is the classical way to estimate the level of supercoiling generated during transcription elongation. The formation of alternative DNA conformations might expose specific DNA binding sites and engage transcription factors, as occurs on the human *MYC* promoter, the best studied example of this regulatory mechanism.[64]

Single-molecule fluorescent in situ hybridization approaches, which allow individual RNA molecules to be measured, have shown that transcription is an intrinsically noisy process.[65] Cell-to-cell variability in gene expression is likely to be dangerous in case of short-life, low-abundance mRNAs of key genes and should be minimized.[66,67] Even if the same promoter in different cells received a signal at the same time, there would be cell-to-cell variability due to the stochastic recruitment of transcription factors and engagement of RNA polymerase. It was shown that due to activated transcription, non-B DNA could form in vivo as far as 1.5 kb upstream of the promoters (**Fig. 2c**). This unusual structure recruited transcriptional factors essential for the fine and tight regulation of *MYC* proto-oncogene output. When this real-time feed-back system is compromised, cells exhibit striking cell-to-cell heterogeneity in MYC levels that could predispose to disease.[53,66,67] In addition to promoting synchronous patterns of gene transcription among groups of cells, it is possible that this mechanism might also help

to equilibrate transcript levels for genes encoding subunits of protein complexes.

Though a similar regulatory mode has also been reported on the *USP29* gene, it is still unknown how widely this cooperation between DNA topology and DNA conformation-sensitive proteins might be used in cells.[68] There are no reasons to believe that *USP29* and *MYC* are special. Considering that abnormal oncogene expression is a common feature of malignancy, the deregulation of the regulatory pathway described above might occur at the promoters of oncogenes and tumor suppressors.[8,69] Recent genome-wide approaches have been used for a fine-analysis of DNA topology in human cell lines revealing that dynamic supercoiling transmitted at least 2 kb upstream from transcription start sites is a characteristic of virtually every transcribed gene (**Fig. 2A**).[39,70] High-resolution mapping of single-stranded DNA also revealed that these structures are a more frequent genomic feature than previously thought.[44] These findings highlight that transcription is inevitably coupled with dynamic perturbations of the double helix and that such perturbations may have the capacity of regulatory feedback in real time.

Negative supercoiling transmitted into the upstream promoter region may also stabilize chromatin fiber by reducing nucleosome mobility.[56] A stable, dominant configuration of promoter chromatin will mask particular sites preventing binding of sequence-specific transcriptional factors that do not participate in the ongoing transcriptional program (**Fig. 2B**, left). Again, this type of feedback might decrease stochastic patterns of transcription by reducing the number of unwanted activation and/or repression events.

Finally, negative supercoiling upstream of transcription start sites could be reinforced if promoters in divergent orientation are transcribed, providing an additional level of regulation (**Fig. 2D**). Divergent promoters represent more than 10% of all human genes, but what is more important is that transcription initiation even from the single promoter is not obligatorily unidirectional. By using different experimental techniques, it was shown that most active promoters support divergent initiation with productive elongation efficiently occurring in the bodies of the coding genes.[71,72] The frequency of this promoter arrangement suggests that it might be used in regulatory pathways.[25] In model systems it has been demonstrated both in vitro and in vivo that supercoiling generated between divergently transcribing RNA polymerases[18,53] is high enough to result in non-B DNA formation, imposing real-time co-regulation of transcription activity as discussed above. Negative supercoiling could also directly facilitate DNA melting during open complex formation. This regulatory mechanism could help to bypass multiple abortive initiation events and to regulate—inhibit or promote—transcriptional noise due to these rate-limiting steps.[19]

DNA Topology and Genome Architecture

It was realized a long time ago that enhancers which may reside at a large linear distance away from the promoter are required for proper gene expression. Master transcription factors bind enhancer regions and by recruiting Mediator activate much of the gene expression programs necessary for cell identity. Recent studies show that active promoter-enhancer communication is accompanied by looping of the intervening DNA in the chromatin complex to juxtapose the enhancer and the target promoter (**Fig. 1D**). The mechanism(s) that establishes this proximity largely remain undescribed, though available data suggest that inter-nucleosomal interactions involving the histone tails are important.[73]

Transcriptome analyses by RNA deep-sequencing show that many enhancers are transcribed.[74] The function of RNA transcripts derived from enhancer sites (eRNA) is unknown, but the transcripts and/or ongoing transcription are required for enhancer action: eRNA has been used as a marker of active enhancers.[75,76] Efficient sliding of interacting chromatin fibers which brings together enhancer and promoter require dynamic rearrangement of chromatin structure. The simple act of RNA polymerase II transcription is sufficient to alter the local chromatin environment and this process likely reflects dynamic supercoiling emanating from the transcribed enhancer independent of gene transcription (**Fig. 2E**).[77-79] Contraction of decondensed chromatin due to "depletion attraction forces" could also contributes to the loop formation.[80] In favor of this idea, it was shown that formation of a chromatin loop topologically isolating the enhancer from the target promoter is sufficient to inhibit enhancer's activity in vivo.[81] Involvement of DNA topology in transcription regulation through enhancers was also elucidated from studies which have shown the presence of topoisomerases at enhancer regions.[82,83]

Dense complexes of DNA and transcription factors in the vicinity of promoters and enhancers likely require a complex architecture of multiple interactions between molecular partners. It is plausible that many of these interactions will be not permissive without strongly bent, twisted or otherwise stressed double stranded DNA, suggesting that cells have evolved a way to modulate the stiffness of DNA.[84] Strategic placement of non-B DNA forming sequences, which could flip into the more flexible conformation in comparison with double helix, might be a means to defeat the rigidity of DNA.[64] Accordingly, in silico analyses showed a high enrichment of these sequences at regulatory regions of the genome and, notably, at the promoters of a wide range of oncogenes.[85-88] In addition, several conformation-sensitive proteins with regulatory function bind and stabilize non-B DNAs, suggesting an important role of these structures in transcriptional output.[64] Another way to make genetic processes more tolerant to the constrained DNA topology is weakening the DNA through introducing double strand breaks or nicks.[84] Locally targeted DNA damage mediated by topoisomerases, which induce a permissive chromatin setting, has been implicated in transcriptional activation by nuclear hormone receptors.[89-91]

In recent years the combination of advanced imaging techniques and new molecular approaches revealed that the eukaryotic genome is organized into sub-megabase loops, termed topological

domains.[12] The domain organization is invariant between different cell types, suggesting that there is no direct relevance to the differential transcriptional activity or development programs. Domain bases are enriched for the proteins such as CTCF and cohesin which are known for their potential to act as a supercoiling boundary. The purpose of such universal large-scale chromatin organization is still to be uncovered, but recent findings hint to the importance of the DNA topology in possible function of these domains. It was shown that topoisomerases facilitate the expression of a large number of long genes with sub-megabase length.[49] Topoisomerase inhibitors affect the expression of long genes in all investigated cell types to date, implying that this DNA topology-dependent effect is common to all mammalian cells. The data rule out any secondary effects on inhibition such as DNA damage and formation of covalent complexes and suggest that topoisomerases have distinct effect on transcription depending on the topology of domain which hosts each particular gene.

The factors that create and maintain this domain organization are presently unknown. CTCF and cohesin might be involved in establishment or maintenance of topological domains in the mammalian genome as their binding sites are enriched at the domain boundaries.[12] However, in a recent study of the topological organization of the genome, it was surprisingly observed that the topological domain structure remains intact in the human cells even after cohesin and CTCF depletion or destruction.[92] Though the proteins responsible for domain formation and maintenance remain to be fully enumerated. Two recent papers suggest that DNA topology itself may be an important player. By using dynamic simulations, it was shown that including supercoiling into models of topological domain organization can qualitatively and quantitatively reproduce experimental 3C data obtained in eukaryotic cells.[93] Another work points to supercoiling and transcriptional activity as critical determinants of domain formation in bacteria. It was revealed that bacterial genome is composed of regions with increased frequency of contacts, highly reminiscent of topological domains in eukaryotic chromosomes.[79] Overall these studies suggest that individual topological domains in eukaryotic cells could be transcription- and topoisomerases-dependent and composed of supercoiled regions forming plectonemes.

Conclusion

In the last few years, new approaches have reinforced the strong relationship between DNA topology and transcription. Together, they suggest that DNA topology provides an additional level of transcriptional regulation and must be precisely controlled. Our understanding of this phenomenon is far from being fundamental and demands the aggressive development of innovative experimental approaches. Exciting times for this area of research are coming.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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